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Evidence of Equine Viral Arteritis (EVA) infection in horses of Serbia

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Equine arteritis virus (EAV) is the causative agent of equine viral arteritis (EVA) and one of the major viral pathogens of horses. EAV is an Arterivirus belonging to the Arteriviridae family in the order Nidovirales. EVA is a respiratory and reproductive disease of horses that occurs worldwide. The vast majority of EAV infections are subclinical, but acutely infected animals may develop a wide range of clinical signs including pyrexia, depression, edema, conjunctivitis and respiratory distress. The direct consequences of EVA outbreaks are financial losses mainly due to abortions of pregnant mares and death of young foals. Following primary EAV infection, up to 70% of the stallions will carry the virus in their reproductive tract sometimes for years and will shed the virus in their semen. Several studies have shown that EAV infection has occurred among horses in North and South America, Europe, Australia, Africa, and Asia. Interestingly, EAV infection prevalence in horses varies between countries and horse breeds. In order to determine equine viral arteritis (EVA) prevalence among the 5000 horses housed in the Vojvodina region of Serbia, 429 sera from non-vaccinated horses have been collected. Serological analysis of equine sera, collected from 2013 and 2014, was performed using virus neutralization test (VNT) as described by the World Organization of Animal Health (OIE). So far sera of 156 horses coming from 10 different stud-farms of the Vojvodina region have been tested. The population tested was composed 86 stallions, aged between 1 and 26 year old, and 70 mares ranged from 1 year old to 23 year old. The mean age of the population tested was 9,9 year old. Our preliminary results indicated that 121 sera were negative (77,60%), thirty three were detected positive (21,15%), two sera were cytotoxic (1,25%). Among the positive sera fifteen (45,45%) exhibit an antibody titer range from 4 to 16, ten (30,30%) sera exhibited a titer ranged from 24 to 96 and eight (24,24%) sera had a titer above 128. Moreover, 9 out of 10 stud farms that have been included in this survey exhibited positive horses for EAV showing that EAV is circulating in the horse population kept in the Vojvodina region. So far, only one seropositive stallion has been found positive for the presence of the virus in his semen. Phylogenetic analysis performed on the 3 kb sequence encoding the structural proteins of the virus shows that this isolate seems different from those described in the literature so far.

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Occurrence of multiple abortions due to *Salmonella enterica* serovar *abortus equi* infection

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Salmonella enterica serovar *abortus equi* (*S. abortus equi*) is a host-adapted organism known to cause abortion in mares and other clinical syndromes in foals. Re-emergence of *S. abortus equi* was identified in Argentina in 2011. Since then several outbreaks of abortion associated with this bacterium had been detected. This study describes the features of an extensive abortion outbreak caused by *S. abortus equi* infection in a polo pony breeding farm located in Buenos Aires province, Argentina. The population at risk consisted of 120 pregnant mares managed in three groups (40 mare/group). They had unrestricted access to pasture. Even though the vaccination program included vaccines against Equine Herpesvirus 1 (EHV-1) and *Rhodococcus equi*, it did not contemplate *S. abortus equi* bacterin. The outbreak of *S. abortus equi* abortion occurred among recipient mares derived from five different embryo transfer centers, with an index case reported on April 4 and the last registered case so far was on July 27, 2015. Abortions took place with no premonitory clinical signs, and the resulting attack rate was 24.2% (29/120 pregnant mares aborted). Nine aborted fetuses and placentas were submitted for necropsy and laboratory diagnosis. Samples from lung, thymus, spleen, liver and placenta resulted negative for Equine Herpesviruses (EHV-1 and EHV-4) and Equine Arteritis Virus by virus isolation and polymerase chain reaction (PCR) in all cases. However, a gram-negative motile bacterium sensitive to a broad range of antibiotics was isolated in pure culture from several tissues collected from all fetuses, and subsequently identified as *S. abortus equi* (4,12: -: e, n, x). Serological diagnosis of *S. abortus equi* was performed in all in-foal mares, and those that showed high antibody titers were treated with Sulfamethoxazol-Trimethoprim (25mg/kg, q12h for 30 days). In addition, in-foal mares were segregated into smaller groups ($\leq$15 mares/group) according to their age of gestation, and were vaccinated with a commercial *S. abortus equi* vaccine (two boosters with a 21-day interval) followed by two additional boosters using an autogenous *S. abortus equi* bacterin. Even though two new cases of abortion occurred right after the treatment was implemented, the outbreak was successfully controlled and no additional cases were reported. The source of the outbreak could not be elucidated due to the diverse origin of the animals and to the absence of reported cases from other farms linked to the affected premise. However, occurrence of *S. abortus equi* associated abortions was reported in other nearby locations. In light of this, a greater awareness of *S. abortus equi* infection as a potential cause of widespread abortions is required among equine practitioners and breeders. Thus, breeding farms need to take into consideration appropriate biosecurity and preventive measures to reduce the risk of abortion outbreaks caused by this agent.

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Salmonella enterica serovar Abortusequi as an emergent pathogen causing equine abortion in Argentine

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Salmonellosis is an infectious disease that affects humans, mammals, reptiles and poultry. It is produced by *Salmonella* spp. which is a gramnegative bacillus of 0.7-1.5 x 2-5 μm, facultative anaerobes and nonsporulating. *Salmonella enterica* serovar Abortusequi (*Salmonella* Abortusequi) is a serovar adapted to the host producing abortion in mares. If the animals do not abort,

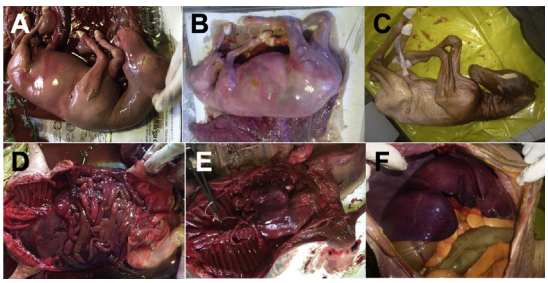

Figure 1. Fetuses with different ages (A,B and C) and signs of septicemia and friable organs at necropsy (D, E and F)

the neonates could born infected showing septicemia and polyarthritis. *Salmonella* Abortusequi was isolated from abortions in 1948 for first time in Argentine. Between 1948 and 1994, abortion outbreaks, mares with reproductive pathologies and goals with septicemia and polyarthritis were described. Nowadays, the worldwide prevalence of paratific abortion is low but in our country, the disease has been reported several times since 2011. The aims of this study were isolated and characterized *Salmonella* Abortusequi from equine fetuses. Six fetuses with different ages were processed in The Laboratory of Infectious Diseases (LEEI) of Facultad de Ciencias Veterinarias, Universidad de Buenos Aires. The fetuses were from two farms of Buenos Aires province (25 de Mayo and Trenque Lauquen cities) where have been more than 40 aborts. Necropsy, bacteriological and histophatological studies were done. Samples of different organs, as stomach contents, liver, spleen, lungs, kidneys, jejunum, colon and cecum were cultured in Xylose Lysine Deoxycholate agar (XLD), blood agar and Selenito broth for 24 h at 37°C. Bacteria were identified by biochemical test and PCR. Antibiotic susceptibility of the strains was performed by the agar diffusion disk method as outlined by the National Committee for Clinical Laboratory Standards (CLSI) using chloramphenicol, florphenicol, tetracycline, ampicillin, cephotaxime, gentamicin, ciprofloxacin, enrofloxacin, streptomycin and trimethoprim/sulfamethoxazole. All the fetuses showed signs of septicemia and friable organs at necropsy but those lesions were more intense in younger animals. Autolysis was observed in most of organs and cell edema and fat damage were observed in liver by histological studies. Lactose and sulfidric negative colonies were isolated from all samples in XLD. Biochemical test were performed by *Salmonella* sp identification. PCR was carry on from Selenito broth and *invA* gen was detected in all cultures. The isolates were identified as *Salmonella* Abortusequi by White-Kauffman-Le Minor schema. All six isolates were sensible to the antibiotics used. Epidemiological characteristics, macroscopic and microscopic lesions, molecular detection and *Salmonella* Abortusequi in purity isolation from all samples allowed identified those *Salmonella* outbreaks. Large economical loses were generated by the large number of lost foals and treatment costs. Previously, infectious abortions were associated with viral etiology as Equine Herpesvirus-1. However, epidemiological data of the last years show an increasing relevance of *Salmonella* Abortusequi in reproduction problems and it could be considered as an emergent pathogen in Argentine. In conclusion, paratific abortion should be suspected in any abortion and both viral and bacterial diagnosis always must be done in all cases to establish appropriate prophylactic and therapeutic measures. We propose to study the clonal relation between strains to understand the epidemiology of the disease in our country.

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Determination of whole-genome sequence of *Salmonella* Abortusequi

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Equine paratyphoid is an infectious disease specific to the family Equidae and caused by *Salmonella* Abortusequi. It is a designated notifiable disease in Japan. Abortion or multiple abscesses are major signs of infection, and the disease is diagnosed by detecting the etiological agent or by serodiagnosis. However, these methods are problematic in terms of sensitivity or specificity: for example, cross-reactivity to *Salmonella* Typhimurium can occur in serodiagnosis. In addition, conventional molecular epidemiological methods have insufficient resolution to discriminate the strains isolated in Japan. To search for a way of solving these problems, we determined the whole-genome sequence (WGS) of *Salmonella* Abortusequi. L-2508, which was isolated in 1987 in Japan, was used as representative strain of *S.* Abortusequi to determine the complete genome sequence by using next-generation sequencing, optical genome mapping, and a gap-closing technique. The size of the genome of L-2508 was 4,738,978 bp; the GC content was around 52%; and 4710 ORFs were detected. A phylogenic tree analysis based on core-genome single nucleotide polymorphisms (SNPs) revealed that *S.* Abortusequi was genetically close to *Salmonella* Choleraesuis and *Salmonella* Paratyphi C. There were 136 pseudogenes; this number was similar to that of *S.* Choleraesuis (host-adapted serotype) and was half as many as that of *S.* Dublin (host-restricted serotype). The WGS of L-2508 was compared with those of other serotypes of *Salmonella* spp. A region unique to *S.* Abortusequi that seemed to be derived from a bacteriophage was detected. Comparison of the presence of known virulence factors in *Salmonella* spp. between *S.* Abortusequi and *S.* Typhimurium detected no *S.* Abortusequi-specific gene. On the other hand, several pathogenic genes were observed only in *S.* Typhimurium. This result suggests that these *S.* Typhimurium genes might be useful for developing a serological method to discriminate infections with the two organisms. The draft whole-genome sequences of 24 strains isolated in various geographic areas and at various times were also determined. Phylogenic tree analysis based on core-genome SNPs was performed on these strains and on L-2508. There were 1316 SNPs among the strains. A difference of about 1100 SNPs was observed between Japanese and foreign strains, whereas only a 61-SNP difference was observed among the Japanese strains. There were two lineages among the Japanese strains. Our results suggest that SNP-based molecular epidemiological methods might be useful for discrimination among Japanese endemic strains.

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Diagnostic epidemiology of nocardioform placentitis and abortion in Kentucky, 1991-2015

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Equine nocardioform placentitis & abortion (NPA) first recognized in Kentucky in the 1980's, is caused by a nocardioform actinomycete bacteria. *Crossiella equi* was first identified in equine placental